

WHAT IS CLAIMED IS:

1 1. An integrated plasmid comprising a biotin synthase
2 gene, an assistant DNA sequence for the integration of said
3 plasmid into a host genome, a promoter sequence, and a
4 selection marker.

1 2. The integrated plasmid as claimed in claim 1,
2 wherein the biotin synthase gene is derived from
3 *Saccharomyces cerevisiae* or *Candida utilis*.

1 3. The integrated plasmid as claimed in claim 2,
2 wherein the biotin synthase gene of *Candida utilis* comprises
3 the nucleotide sequence of SEQ ID NO: 1.

1 4. The integrated plasmid as claimed in claim 1,
2 wherein the assistant DNA sequence is a *Candida utilis*
3 fragment selected from the group consisting of NsiI-BamHI
4 18s rDNA, URA3 DNA, and HIS3 DNA.

1 5. The integrated plasmid as claimed in claim 1,
2 wherein the selection marker is a cycloheximide-resistant
3 gene.

1 6. The integrated plasmid as claimed in claim 1,
2 wherein the promoter sequence is selected from the group
3 consisting of pL41 promoter of *Candida utilis* and pADH1
4 promoter of *Saccharomyces cerevisiae*.

1 7. The integrated plasmid as claimed in claim 1,
2 wherein the integrated plasmid is selected from the group
3 consisting of:

4 (a) pMCC21 (having the configuration of restriction
5 sites in FIG. 6);

6 (b) pMCC31S (having the configuration of restriction
7 sites in FIG. 8);

8 (c) pMCC32H (having the configuration of restriction
9 sites in FIG. 9);

10 (d) pMCC33U (having the configuration of restriction
11 sites in FIG. 10);

12 (e) pMCC35U (having the configuration of restriction
13 sites in FIG. 11);

14 (f) pMCC36H (having the configuration of restriction
15 sites in FIG. 12); and

16 (g) pMCC38S (having the configuration of restriction
17 sites in FIG. 13).

1 8. A method for preparing a yeast with high biotin-
2 productivity, comprising the steps of:

3 constructing an integrated plasmid comprising a biotin
4 synthase gene, an assistant DNA sequence for the integration
5 of said plasmid into a host genome, a promoter sequence, and
6 a selection marker;

7 linearizing said integrated plasmid;

8 transforming said linearized integrated plasmid into a
9 yeast; and

10 recombining the biotin synthase gene with the yeast
11 genome.

1 9. The method as claimed in claim 8, wherein the biotin
2 synthase gene is derived from *Saccharomyces cerevisiae* or
3 *Candida utilis*.

1 10. The method as claimed in claim 9, wherein the
2 biotin synthase gene of *Candida utilis* comprises the
3 nucleotide sequence of SEQ ID NO: 1.

1 11. The method as claimed in claim 8, wherein the
2 assistant DNA sequence is a *Candida utilis* fragment selected
3 from the group consisting of NsiI-BamHI 18s rDNA, URA3 DNA,
4 and HIS3 DNA.

1 12. The method as claimed in claim 8, wherein the
2 selection marker is a cycloheximide-resistant gene.

1 13. The method as claimed in claim 8, wherein the
2 promoter sequence is selected from the group consisting of
3 pL41 promoter of *Candida utilis* and pADH1 promoter of
4 *Saccharomyces cerevisiae*.

1 14. The method as claimed in claim 8, wherein the
2 prepared yeast with high biotin-productivity is useful as
3 feed additives, food additives, or cosmetics.

1 15. A method for producing biotin, comprising:
2 providing the yeast with high biotin-productivity of
3 claim 8; and
4 culturing said yeast in a nutrient medium, and
5 recovering biotin from the culture broth.

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- 1 16. The method as claimed in claim 15, wherein the
- 2 recovered biotin is useful as feed additives, food additives,
- 3 or cosmetics.